Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2016

Volume 27

Projects with a primary purpose of: Translational Applied Research – Other Human Disorders and Non-Regulatory Toxicology/ecotoxicology

Project Titles and keywords

1. **Experimental surgery in pigs under terminal anaesthesia**
    - Pigs, surgery, model

2. **The use of in vivo models for drug discovery**
    - Inflammation diabetes pain

3. **Zebrafish: an alternative model for the safety assessment of agrochemicals**
    - toxicology; agrochemical

4. **Hazard of Nanomaterials and related substance**
    - Nanomaterials, particulates, safety classification

Project 1	**Experimental Surgery in Pigs Under Terminal Anaesthesia**	
Key Words (max. 5 words)	Pigs, surgery, model	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The general purpose of this application is to allow surgical studies to be conducted on pigs under general anaesthesia from which they will not be allowed to recover. In the first instance, operations will involve kidney and liver removal in studies examining methods intended to improve transplanted organ function in people. (The current shortage of organs for transplantation in the UK arises in part from potentially suitable organs becoming damaged in the period between donation and transplantation). Later studies will provide preliminary or "proof-of-concept" information that may indicate the need for further investigations conducted under separate licenses. It is also intended to study and improve the anaesthesia and monitoring of pigs under anaesthesia for this kind of surgical study.	

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project will use pigs to improve the current methods used to preserve organs for donation and so increase the number available for transplantation to human patients. This will: a) reduce the number of donated organs required; and b) reduce the number of people dying from kidney and liver failure in the UK In addition, the meticulous management of the animals, in conjunction with the collection of an extensive range of physiological data, will improve the management of animals on this and future studies.
What species and approximate numbers of animals do you expect to use over what period of time?	Pigs 120 over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	In a small number of cases, animals may be initially treated with a drug that could improve the outcome of the study. Then they will be anaesthetised. All the animals will be anaesthetised only once and will be killed under anaesthesia without being allowed to recover. They will not experience anything more unpleasant than treatment with a drug - which is not expected to have any adverse effects – followed by a single injection in their muscles to sedate them in preparation for general anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Whilst much information on techniques for preserving transplanted organ function can be obtained from isolated organs, such organs have to be retrieved from the whole anaesthetized animal in the first instance. The effects of some of the techniques proposed cannot be evaluated in anything but the whole animal.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The need for ongoing animal use will be assessed after every study. This will ensure that the rate of improvement of experimental design and protocols is optimized, and that the study will be stopped, if it is determined unlikely to be successful in the long-term. Providing high quality anaesthetic care will improve

	the quality of information collected in the study and by increasing study power, reduce the number of animals required to achieve statistically significant end-points. Imaging and repeated tissue sampling will also reduce the variability between animals and help reduce animal use. Statisticians will be involved in the animal-by-animal analysis of experimental progress. These three measures will ensure that unwarranted animal use is minimized.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Pigs are an established model for studies of human conditions, including renal and liver transplantation because the organs are similar in size and anatomy to those from humans. The applicant has extensive experience in the prolonged management of anaesthetized pigs. During each study, the methods used will be assessed on an animal-by-animal basis and improved if opportunities for further refinement are identified. At the conclusion of each study, details of these improvements will be published to assist other researchers. All animals will be anaesthetized for all studies, from which they will not be allowed to recover. Consequently, they will typically experience nothing more unpleasant than a single injection that in some pigs may cause mild and momentary discomfort.

Project 2	**The use of in vivo models for drug discovery**	
Key Words (max. 5 words)	Inflammation diabetes pain	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of this project is to test drug efficacy in animal models of specific diseases. This information will be invaluable in the development of novel compounds for the treatment of these diseases.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	All of the disease areas that we have focussed upon are major causes of morbidity and mortality worldwide. This project is an important step on the path to discovering new drugs to treat these conditions. The models of disease (including respiratory disease, diabetes, psychosis and pain) involve complex biological responses and therefore cannot be replicated in other ways. We will use these animal models to aid the development of novel compounds for the treatment of these diseases.	
What species and approximate numbers of animals do you expect to use over what period of time?	We have chosen to use rodent models (mice and rats) as their inflammatory and immune systems and cell biology are extremely well documented. In addition, our researchers have considerable experience and expertise using rodent models. We propose to use approximately 260 rats and 420 mice	

	per year, for 5 years, however this number is dependent on client demand for the particular models.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The expected adverse effects are listed for each specific model. The disease models outlined in this project license produce responses that mimic certain aspects of the disease processes seen clinically, but without progressing to the terminal and severe stages of the disease processes found in humans. Adverse effects may include some signs of discomfort such as listlessness and hunching following intra-peritoneal injections and intra-tracheal instillations. These signs should be transient in nature. For the diabetes models, repeated blood sampling via the tail vein may cause momentary discomfort. For the pain models, surgical procedures may result in short-term discomfort which will be minimized by careful husbandry, including providing soft paper bedding and soft food diet. All animals will be killed by schedule I method at the end of each model.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The models of disease (including respiratory disease, diabetes, psychosis and pain) involve complex biological responses and therefore cannot be replicated in non-animal experiments.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In addition to our animal research facilities, we run a large number of standard cell-free and cell-based assays and toxicology screens. As such, we have the capacity to carry out extensive analysis of novel compounds in our standard lab-based experiments before they are put forward for testing in animals. The models that we will use are all “industry standard” models that are well described. As such, we hope to use a minimal number of animals to complete these studies.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most	We have chosen to use rodent models as their inflammatory and immune systems and cell biology are extremely well documented. In addition, our researchers have considerable experience and

refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	expertise using rodent models. The disease models outlined in this project license produce responses that mimic certain aspects of the disease processes seen clinically, but without progressing to the terminal and severe stages of the disease processes found in human. Animals will be housed in cages with environmental enrichment and handled by experienced personnel. Good husbandry and care practices based on veterinary advice will be used for all animals which have undergone experimentation and the animals will be observed daily for adverse effects to be detected at an early stage, and steps taken to minimise them.

Project 3	**Zebrafish: an alternative model for the safety assessment of agrochemicals**	
Key Words (max. 5 words)	toxicology; agrochemical	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Prior to marketing, agrochemicals (e.g. pesticides) must be tested for their potential to harm humans who may be inadvertently exposed. This is normally conducted in mammals (e.g. rats and rabbits), however, embryolarval zebrafish are a credible alternative model for human toxicity assessments due to their close physiological and genetic similarity with humans. In addition, their small size (<5mm) means larval zebrafish can be used to test for toxicity at an earlier stage in the development process. Consequently, this project has the main aim of evaluating the embryolarval zebrafIsh as an early stage model for assessing new agrochemical products for their potential to cause side effects in humans, in this case regarding harm to the unborn child (developmental toxicity).	
What are the potential benefits likely to derive from this project (how science could be	The regulatory requirements for developmental toxicology are extensive, requiring several in vivo (in live animals) tests in mating and pregnant animals	

advanced or humans or animals could benefit from the project)?	(usually rats and rabbits), and subsequent assessment of toxic effects in parents and foetuses. These tests require Large numbers of mammals, are extremely expensive and time consuming to perform, and thus are typically performed late in the development of a new agrochemical. However, issues at this late stage can result in the termination of a project after considerable investment, not least in animals used in the many studies performed prior to this point. Consequently, there has been much interest in the use of the zebrafish as an alternative model to be able to test this potential toxicity earlier in compound development to ultimately help improve the quality and effectiveness of new agrochemicals entering mandatory regulatory testing.
What species and approximate numbers of animals do you expect to use over what period of time?	We estimate that we will - use approximately 17000 embryolarval zebrafish (age: 5 days post fertilisation) over the duration of this project licence (5 years). This estimate is based on evaluating an established protocol for embryolarval zebrafish developmental toxicity (validated for use on human pharmaceuticals) for use on agrochemicals by testing approximately 70 compounds spanning a range of different mechanisms of action.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	One protocol is proposed, .which is based upon the use of a method previously validated for testing human pharmaceuticals. We will evaluate this method for testing agrochemicals for their potential to cause developmental toxicity in humans, and to subsequently apply this method to test new agrochemical products for this unwanted adverse effect (subjected to a successful outcome to the evaluation exercise). Agrochemical testing will be by immersion (in very dilute solutions) after which animals are assessed for the signs of developmental abnormalities. For this we use a detailed visual scoring system that allows us to judge whether exposure to the agrochemical is resulting in abnormal development or not. The aim for this work is to undertake early side effect testing in a lower vertebrate (embryolarval fish) rather than in a rat or rabbit, as a step towards replacement of mammalian

	testing and with the ultimate goal of working towards non- animal models. Although the aim is to assess sub lethal indicators of toxicity, there may be mortalities, as most of the compounds used in the evaluation have not been tested on fish before. However, experience with human pharmaceuticals suggests that this will account for just 6% of the embryolarvae exposed. Similarly, although the licence has an overall rating of severe (due to the unknown toxicological effects of some of the test compounds), previous experience suggests that <75% of animals will not undergo a severe procedure. All animals are humanely killed immediately after assessment (5 days old).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Living whole animals are ultimately necessary to accurately recreate the complex development of organs and structures in the growing organism. In mitigation, the overall goal is to reduce (ultimately unnecessary) mammalian testing on agrochemicals that may fail later in compound development due to a poor side effect profile. The data generated are also the first towards being able to build computer models which, in future, may negate the need for animal testing altogether.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Every aspect of experimental design is geared towards reducing animal use: we routinely use statistics to determine the minimum number of animals needed; often use shared control groups; maximise information gained per animal used; and strive to combine multiple measurements in the same animals wherever possible. The overall purpose is to reduce the number of tests undertaken unnecessarily on mammals with agrochemicals that will eventually fail due to a poor side effect profile. The generation of sufficient and robust data using the zebrafish (through validation and testing) for the assessment of side effects related to developmental toxicity could allow the zebrafish to be used to potentially reduce and or replace the requirement for higher vertebrate testing.

3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Zebrafish are amongst the simplest vertebrates which are considered biologically representative of mammals including humans. Our project involves observation after treatment with agrochemicals, and is conducted in very young animals (—5 days old, 3 mm long). Consequently, adverse effects are most likely associated with unpredictable agrochemical properties. To minimise suffering we continually review dosing levels as more information is gathered; share data between different tests and users; and frequently monitor animals and humanely kill them at the minimum level of suffering that meets the aims of the test. Due to the use of test chemicals where there are often no information available on their toxicity, death as consequence of exposure cannot be ruled Out (hence the severe classification). However, as stated a number of control measures are in place such that this will only occur in the minimum numbers of animals possible.

Project 4	**Hazard of Nanomaterials and related substance**	
Key Words (max. 5 words)	Nanomaterials, particulates, safety classification	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
	x	Regulatory use and routine production
	x	Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Engineered nanomaterials and their associated micron scale substances used in nanotechnology products come in many shapes, sizes, and chemistry. The current approach of animal testing on a substance-by-substance basis is not sustainable for the diversity of materials already being made. The overall aim is to provide data on biological effects on animals, and with in vivo, and ex-vivo data, as well as information on physical chemistry of the materials, to build a predictive computer model that will group nanomaterials by type, and reduce the need to use animals for chemical safety testing in the EU thereafter.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Nanotechnology is identified as a priority for economic growth and prosperity in the EU, and this work will enable safe, responsible innovation. The model will eliminate and reduce several aspects of animal testing in the EU and in countries involved in the consensus building on nanomaterial testing at the Organisation for Economic Cooperation and	

	Development (OECD).
What species and approximate numbers of animals do you expect to use over what period of time?	Mostly fish such as rainbow trout, zebrafish, sticklebacks, tilapia, African catfish, and some mice or rats. The latter work on rodents is mainly to demonstrate that fish data can replace work on mammals. Over 5 years the project estimates use 24,000 fish (including embryos and larvae), 360 mice, and 500 rats.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The project intends to look mostly at physiological and sub-lethal effects using exposures via the water or food. With novel materials there is a risk of unintended or unexpected effects including possible respiratory toxicity in fishes (severe) and irritation to the gut or changes in nutrition in animals fed food containing nanomaterials (moderate). In some experiments the lethal concentration maybe measured (severe). However, computer predictions of hazard, cell culture work, and ex-vivo studies on organs will inform on reducing the amount of animal testing in the moderate and severe categories. Animals will be killed humanely at the end of each experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our overall ethical goal is to build a computer model that is predictive and can replace animal testing for nanomaterials. The predictive tool must be validated with some animal data. The experiments will necessarily use animal models that are accepted in regulatory testing by the EU including rainbow trout, zebrafish, mice and rats. Some of our partners are international and so some work on tilapia and African caffish maybe done, although we will also work on our native freshwater sticklebacks.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We are using some animals now to obtain data to make the computer model, and this will enable a step reduction in animal use in the EU thereafter. Within our work, in vitro studies and ex-vivo organ studies will inform on the materials of concern and enable

	decisions to not test substances further in vivo. Experiments on fish will inform on whether or not rodent studies are needed. Our statistical approach and experimental designs are minimising the use of animals within each type of experiment.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The first protocol in the licence is a lethal range finding experiment with fish that will enable refinement of dosing in any subsequent experiments. The animal models above are selected for practical reasons relating to standardisation of regulatory testing, not for ethical refinement per se. However, measures taken to reduce suffering will include using lower doses that are less toxic, shortening experiments where possible, and withdrawing individual animals from experiments for health reasons.